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with a Potential Role in Tumorigenesis and Cancer  
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## FOREWORD

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N.A. In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

<u>Lisa M. Swankart</u>	22 July 2002
PI - Signature	Date

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## **(5) Introduction:**

One of the deadliest and least understood aspects of cancer is metastasis. Before a tumor can metastasize, individual cells must acquire mutations which down-regulate adhesion to neighboring cells. A number of studies have shown that down-regulating components of the adherens junctions, one of the primary cell-cell adhesion systems, causes increased invasiveness and metastatic potential of tumors (reviewed in (Behrens, 1999)). In adherens junctions, the extracellular domains of cadherins form homotypic interactions with neighboring cells (reviewed in Tepass et al., 2000). The cytoplasmic tail of cadherin interacts with a class of proteins termed catenins, which associate with the actin cytoskeleton. Regulation of adhesion is an important process for cellular rearrangements such as during development and axon neurogenesis. p120 is a candidate molecule that may function as a modulator of cell adhesion through its interaction with the membrane proximal region of the cadherin cytoplasmic tail (reviewed in (Reynolds and Daniel, 1997)). In vertebrates p120 is phosphorylated by tyrosine kinases in response to a variety of growth factors. Correlated with p120 phosphorylation is a breakdown of adherens junctions, rearrangement of the actin cytoskeleton, and a loss of cell-cell contacts. p120 becomes highly tyrosine phosphorylated in metastatic, non-adherent cells. We are studying p120 in *Drosophila* where we can take advantage of the many well-characterized tools in this genetic model system. *Drosophila* is an excellent model because flies have adherens junctions that are highly homologous to vertebrate adherens junctions at the molecular level. In addition, we have identified only a single p120 homolog in flies, and, with the completion of the genomic sequence, we are reasonably certain that there are no others. The objective of this research project is to characterize the role of p120 by generating flies mutant for the p120 gene and characterizing them phenotypically and biochemically.

**Aim I. Intracellular localization and interaction of *Drosophila* p120 with *Drosophila* E-cadherin and other catenins**

**Aim II. In vivo structure/function analysis of Dp120ctn**

**Aim III. Transfer of knowledge gained in *Drosophila* to the study of breast cancer.**

## **(6) Body:**

This project was a collaboration between Rob Cavallo, the original awardee, who received his Ph.D. in December 1999, Gordon Polevoy, PI from May 2000-April 2001, Lisa Swanhart, the PI from August 2001 to June 30, 2002, and Steve Myster, a postdoctoral fellow. Ms. Swanhart became PI in August 2001, after her selection and a one-year no-cost extension were approved by the Agency.

### **Aim 1 Intracellular localization and interaction of *Drosophila* p120ctn with *Drosophila* E-cadherin and other catenins**

The p120 C-terminus was used to generate polyclonal antibodies in both rabbits and rats. On western blots the antibodies are specific for a doublet of 90-100 kDa. These antibodies work in situ on embryos using immunofluorescence confocal microscopy. Dp120 is ubiquitously expressed and localizes to cellular junctions (Fig. 1A). There is also a fair amount of cytoplasmic staining. Interestingly, in early stage embryos a pair of bright staining structures were also identified—these appear to be centrosomes (see below). The pattern of p120 intracellular accumulation closely resembles that we observe with a myc-tagged p120 (Fig. 1B; see below). Later in development, p120 becomes concentrated in axons (Fig. 1C), as does Armadillo. We are continuing to characterize the pattern of p120 accumulation, and are using our null mutant embryos as a control for specificity.

We also generated two p120 transgenes that encode Dp120 with an added N-terminal 6xmyc-epitope. These were used to generate transgenic *Drosophila*—we have 7 lines under control of the ubiquitin promotor, resulting in ubiquitous expression, and one line under the control of the GAL4-UAS system (Brand and Perrimon, 1993). The myc-tagged p120 is recognized both by our polyclonal anti-p120 antibodies (confirming their specificity) and by a monoclonal antibody against the myc-epitope. While different lines are expressed to different levels, the ubiquitin promotor drives expression at levels similar to those of endogenous p120. We used these lines to examine p120 subcellular localization. p120 localizes to cell-cell junctions (Fig. 1B,D), co-localizing with both DE-cadherin (Fig. 1D) and Armadillo (data not shown). The staining is quite similar to that seen with our anti-p120 polyclonal antibodies (Fig. 1B,C), although the level of cytoplasmic staining is lower. We can also detect accumulation of p120 in the CNS using the anti-myc antibody (Fig. 1C). Finally, we used the myc-epitope to immunoprecipitate (IP) p120. It works well for this, and preliminary results suggest that it co-IPs DE-cadherin, Armadillo, and alpha-catenin.

Finally, we generated a p120 transgene as a C-terminal GFP-fusion. The initial transgene lacks the C-terminal 54 amino acids of p120 (for ease of cloning), but we are generating a transgene with a fusion of GFP to full-length p120. These have been used to generate transgenic *Drosophila*— we have

eight lines under control of the ubiquitin promoter, and five line under the control of the UAS-GAL4 system. The GFP-tagged p120 is recognized by anti-GFP antibodies, but is not recognized by our polyclonal anti-p120 antibodies, suggesting that either their epitope is in the C-terminal 54 amino acids that were truncated in cloning or that the C-terminal GFP fusion blocks the epitope by steric hindrance. We used this GFP-tagged p120 to examine p120 subcellular localization both in living and fixed embryos (Fig. 2). It gives a very strong signal in living embryos, and strongly labels cell-cell junctions (Fig. 2A-C). It also localizes to developing tracheae (Fig. 2D), axons of the CNS (Fig. 2E), and to the sense organs of the peripheral nervous system (Fig. 2F). We are currently using the p120 GFP fusion to examine the dynamic behavior of p120 during the morphogenetic movements of embryogenesis, by taking time-lapse movies of these processes using our spinning disc confocal microscope. In addition to these expected locations, we also found p120 in one unexpected place. The p120-GFP accumulates in paired structures within each cell—double labeling with antibodies to the centrosomal proteins centrosomin and gamma-tubulin reveal that these are centrosomes (Fig. 2G). This result is quite surprising, reflecting a novel localization for *p120*. We confirmed that this is not an artifact of the GFP-fusion, as we can see endogenous p120 at centrosomes using our anti-p120 antibodies.

We previously mapped the binding site of fly p120 to the juxtamembrane domain of DE-cadherin. During the course of this work, we initiated a collaboration with Ken Kosik's group at Harvard to study the interaction between a mammalian p120 family member, delta-catenin, and E-cadherin. We found that it also bound to the juxtamembrane region. These data were published in the *Journal of Cell Biology* (Lu et al., 1995), with Rob Cavallo and Mark Peifer as co-authors, acknowledging the support of the DOD.

## **Aim 2 In vivo structure/function analysis of Dp120ctn**

### **Mapping *p120* to a particular region of the 2<sup>nd</sup> chromosome using deficiencies**

The *Drosophila* p120 gene maps to the 41C region of the right arm of the second chromosome. Because of its position at the edge of the heterochromatin, the number of genetic reagents available in the region was small—in particular, when we initiated this analysis there were no P elements in the vicinity of the gene. As the first step toward isolating a mutation in p120ctn, we mapped the gene with regard to several chromosomal Deficiencies in the region (Fig. 3), using a combination of polytene in situ and other techniques. To more finely map the *Dp120* gene, deficiency strains and balanced stocks that contain mutations that also map to the 41C region were obtained from the Bloomington stock center. In preparation for the *Dp120* genetic screen (see below) we determined which of the deficiencies removed the *Dp120* gene (Fig. 3). We chose to use *Df(2R)M41A8* for a genetic screen for lethals in the region (see below), as it was the smallest of the Deficiencies we initially identified that removed *p120* and the heterozygous Deficiency stock is healthy.

One problem that slowed progress is that *p120* is at the junction of euchromatin and heterochromatin, in a region where genes are embedded in repetitive sequences. This region of the genome had not been well studied. We collected (and generated—see below) small Deficiencies in the region. We used these to connect the physical and genetic maps (Fig. 3). To do so, we used GFP-marked Balancer chromosomes to select homozygous mutant embryos from their siblings. DNA was isolated from multiple single embryos and analyzed by PCR using two different sets of primers from *p120* as well as primer sets for several other genes in the region. This allowed us to determine whether *p120* or the other genes were removed by particular Deficiencies (Fig. 3). This allows us to correlate the physical and genetic maps, and also suggested that candidate genes for two of the other complementation groups derived from our screen—*Nipped A* may correspond to *CG2905* and *l(2)NC85* may correspond to *CG2682*. We are currently testing this by sequencing the open reading frame for these predicted genes from homozygous mutant DNA.

We initiated genetic analysis by carrying out a genetic screen for lethal mutations in the region of one of the Deficiencies that removes *p120*, *Df(2R)M41A8*. This screen was based on the premise that *p120*, like other core components of the adherens junction in flies and mammals, would be an essential gene. We screened 6284 chromosomes and isolated 226 lethal lines (results are described below). Subsequent analysis, involving mapping mutations to Deficiency intervals and sequencing the *p120* gene from selected mutants, revealed that none carry a mutation in *p120*. This suggested that: a) mutations in *p120* are not represented in our collection, and/or b) mutations in *p120* are not zygotically lethal. We thus implemented other approaches to abrogate p120 function. First, we designed reagents to target *p120* for inactivation by double-stranded RNA interference (RNAi), an approach which has now been successfully used to inactivate a number of different fly genes (e.g., Kennerdell and Carthew, 1998). We generated dsRNAs against two regions of *p120*. We also made templates to RNAi *ftz*, a positive control used by many groups, and to *armadillo* and *DE-cadherin*, as positive controls for other junctional components. We obtained the expected phenotypes with *ftz*, *arm*, and *DE-cadherin*. In the

case of *p120*, only a small fraction of the embryos were lethal, and most had no abnormalities in their body plans. We suspect that the small fraction that did die represent those killed by the injection procedure. These data thus did not support an essential role for *p120*, but are not definitive.

We also initiated an alternate genetic approach. Recently, the Berkeley *Drosophila* Genome Project (BDGP) reinitiated its search for P-element insertions in new regions of the genome, using a novel P-element that allowed the recovery of insertions in the heterochromatin. One of these, *KG01086*, maps about 8 kb 3' to *p120*, between it and the next adjacent gene, *CG17857*. This insertion is homozygous viable, as it disrupts neither gene. We mobilized this insertion, screening for changes in eye color that might indicate either a transposition event to a nearby site or a deletion beginning in the P element and extending into adjacent DNA. Putative mobilization events were screened by PCR, and four deletions affecting the *p120* gene were isolated. All remove the entire coding sequence, as assessed by PCR, and three of the four do not affect any other genes. To our surprise, all are zygotically viable. Further, we generated homozygous stocks of all of these lines, suggesting that *p120* is not male or female sterile, nor is a zygotic phenotype covered by a maternal contribution. We are currently analyzing the levels and localization of other junctional proteins in the mutants, to see if we can detect a cell biological or biochemical phenotype. Our data are consistent with data obtained in the nematode *C. elegans* by Jeff Hardin and Matt Petit. *C. elegans* also only has a single *p120* relative. These investigators used RNAi to eliminate *p120* function, and found that this does not result in a discernable phenotype. They have, however, found that *p120* RNAi enhances the phenotype of a weak mutation in the worm alpha-catenin homolog. This suggests that *p120* may play an accessory role in adhesion. To further investigate is in *Drosophila*, we are generating embryos that are double mutant for *armadillo* and *p120* or *DE-cadherin* and *p120*, using a range of alleles of both *armadillo* and *DE-cadherin*, including null alleles and weak alleles. This should reveal whether loss of *p120* function enhances the adhesion defects seen in these mutants. We are preparing a manuscript describing these results, which we hope to submit this fall. This manuscript will acknowledge the support of the DOD in this work.

#### **Genetic analysis of the euchromatin-heterochromatin boundary**

In our initial effort to obtain mutations in *p120*, we carried out a molecular genetic and genetic analysis of the 41C region, providing a window on the euchromatin/heterochromatin junction. This region of the genome proved challenging for the BDGP. Due to the repetitive nature of this region, they were not able to fully assemble the sequence of the region, nor were they able to define a contiguous contig of clones. *p120* mapped to the most proximal of the scaffolds assembled (AE002751), with *p120* the most proximal sequenced gene defined. One additional scaffold was assigned to the region (AE002760), which was thought to lie between *p120* and the rest of 2R (which begins with scaffold AE003788). The BDGP had also assigned numerous BAC clones to the 41 region, and had mapped sequence tagged sites (STSs). We used this information as a starting point to carry out additional bioinformatic analysis of this region, to clarify the physical map. We carried out BLAST searches of the Celera shotgun sequence scaffolds with STSs in the region. This identified several additional scaffolds that are likely to map to the region, and allowed us to tentatively order them with respect to the scaffolds known to map to this region. We also carried out BLAST searches of the partially sequenced BDGP BAC clones with both STSs and genes from these scaffolds. These data allowed us to derive a proposed physical map of the region (Fig. 4). Our proposed map suggests that the current set of BACs spans the entire region, and provides a possible BAC tiling path to provide complete sequence of the region. The BDGP is currently filling gaps and finish sequencing this region.

We next examined the annotated sequence of these scaffolds to determine how many genes we might expect in the region. Our analysis suggested that initial Celera/BDGP annotation included several retrotransposon remnants among the CGs. Most of the rest of the CG's appear to be bona fide genes, with clear orthologs or sequence relatives in other species (Table 1), some of which have known or inferred functions. Gene density through much of the region is quite low, with some of the scaffolds containing no annotated CGs. The region can be roughly divided into four parts, based on gene density. In the most proximal region, the average gene density is quite low-- one gene per 27.8 kb, relative to a genome-wide average of one gene per 9 kb. Next is a region of more than 315 kb containing no predicted genes. Next most distal is a long region with low gene density. Gene density then increases fairly abruptly in the most proximal scaffold to a density similar to that of most of the euchromatic genome. A similar organization of the heterochromatin-euchromatin junctions was previously observed at the base of the X and of 2L, which were analyzed by the BDGP (Adams et al. 2000). In addition, a number of genes on the most proximal scaffolds (e.g., *LD05623*, *p120ctn*, and *NippedB*) and in the more proximal region of the more distal scaffolds (e.g., *dd4*, *Ogt*, *CG30437*, and *CG30438*) are interrupted by large introns, a feature that is less frequent in the genome in general.

To obtain a more detailed view of the nature of the sequence of this region of the genome, we carefully examined the nature of the 23 kb of sequence in the vicinity of *p120*. The sequence was analyzed by BLAST, using 1-2 kb segments as queries to search the transposon and repeat databases of the BDGP, as well as the EST, predicted gene and genomic databases. The results were quite striking. The majority of the sequence of the region was composed of repetitive DNA, largely the remnants of various retrotransposons. Two elements, 1360/Hoppel and Narep1/Ine1 account for a large fraction of this sequence. In most cases, only fragmentary elements were present, which were internally deleted or otherwise rearranged. In addition to known elements, other regions were clearly repetitive, though they were not closely related to any known transposon.

Finally, we carried out an F2 recessive lethal screen to identify EMS induced mutations that are lethal over deficiency *Df(2R)M41A8* (aka *Df740*). We screened 6,194 chromosomes and recovered 200 new mutants. We took advantage of three additional deficiency strains that overlap the deficiency used in the screen (Fig. 3): *Df(2R)M41A10* (aka *Df741*), *Df(2R)Nap1* and *Df(2R)345*. Together these four Deficiencies divide the region into a series of Deficiency intervals. Each of the new mutants was tested for complementation against these strains, dividing them into resulted four subgroups (Fig. 3), only one of which could include mutations in *p120*. Mutant strains in the first class complement *Df(2R)741* and fail to complement *Df(2R)Nap1*. In addition, a group of new mutations complemented the other three deficiency strains. Mutations that met these criteria are likely to represent mutations elsewhere on the second chromosome that fail to complement other lethal mutations that have accumulated on the chromosome carrying *Df(2R)740*, which was used in the screen. We further ordered the mutations in this region by using a set of very small deficiencies in the region. We determined that *Df(2R)Nipped D*, *Df(2R)Nipped E*, remove the *p120* gene while *Df(2R)Ae*, and *Df(2R)Nipped C* do not remove *p120*. Thus the smallest Deficiency interval that contains *p120* is the overlap between *Df(2R)Nipped D* and *Df(2R)345* (Fig. 3). We carried out complementation tests between these deletions and the NC mutations in the region. We defined two complementation groups in this minimal region, which are *l(2)41Af*, of which we have at least three alleles, as well as the cloned gene *Nipped B* (Rollins et al., 1999), of which we generated 18 new alleles. One unusual feature of our screen is that a fairly large number of mutations derived from our screen appear to be deletions that remove more than one complementation group. Results obtained in an earlier genetic analysis of this region, which were initially interpreted to suggest complex interallelic complementation patterns, may also be explained by the generation of many deletions by EMS mutagenesis (Hilliker, 1976). EMS mutagenesis does not usually result in a high frequency of deletions—however, perhaps the highly repetitive nature of the DNA in the region in question predisposes to the creation of deletions due to mispairing between adjacent repeats during the repair process. We are currently preparing a manuscript describing the genetics and molecular genetics of the 41C region, focusing on novel features of the genome at the junction between the euchromatin and heterochromatin. We will submit this manuscript for publication this fall, acknowledging the DOD for its support.

### **Aim III Transfer of knowledge gained in *Drosophila* to the study of breast cancer.**

We have initiated a collaboration with the group of Keith Burrige, to examine the function of fly *p120* in cultured mammalian cells. They previously found that over-expression of mammalian *p120* has a dramatic effect on cell morphology, and that this appears mediated in part by an interaction of *p120* with the proto-oncogene *Vav2*, which acts as a GEF for Rho family GTPases (Noren et al., 2000) –similar results have been obtained by the Reynolds lab (Anastasiadis et al., 2000).

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Lu, Q., Paredes, M., Medina, M., Zhou, J., Cavallo, R., Peifer, M., Orecchio, L. and Kosik, K.S. (1999). Delta-catenin, an adhesive junction-associated protein which promotes cell scattering. *J. Cell Biol.* 144, 519-532.

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### Training accomplishments

Rob Cavallo, the original PI of this grant, defended his thesis in December 1999, and received his Ph.D. His thesis was composed in part of the work described above, and thus was supported in part by this grant. Rob presented the work to date at the Era of Hope Meeting in Atlanta in June 2000. A new graduate student, Gordon Polevoy, took over from Rob on the project, and this transfer was approved by the relevant officials of the USAMRMC. As noted above, Mr. Polevoy graduated with an M.S. degree in April 2001.

### Appendix 1: Key research accomplishments.

- a) We generated two different polyclonal antibodies which recognize p120 both on immunoblots and in situ in fixed embryos. P120 localizes to the cytoplasm and the cell-cell adherens junctions.
- b) We generated two different myc-tagged p120 transgenes under the control of different promoters. Both are expressed and the ubiquitin promoter drives expression at levels similar to that of the endogenous promoter. We used the epitope tag for in situ localization, confirming our work with the p120 antibody. We also used it to immunoprecipitate p120 and in preliminary experiments have found that it co-IPs with other adherens junction proteins.
- c) We generated two different GFP-tagged p120 transgenes under the control of different promoters. Both are expressed and allow us to visualize p120 in living embryos.
- d) We mapped *p120* to a small region of the right arm of the second chromosome and correlated the genetic and physical maps in the region.
- e) We isolated 200 lethal and visible mutations in the *Dp120* region.
- f) We mobilized a P element in the vicinity of *p120* and used it to generate null mutations in *p120*. To our surprise, these mutations are viable and fertile. We are currently analyzing them for genetic interactions with mutations in other adherens junction components.

### Appendix 2: Reportable outcomes in the entire 3 years.

#### Publications supported in part by this grant

Lu, Q., Paredes, M., Medina, M., Zhou, J., Cavallo, R., Peifer, M., Orecchio, L. and Kosik, K.S. (1999). Delta-catenin, an adhesive junction-associated protein which promotes cell scattering. *J. Cell Biol.* 144, 519-532.

#### Presentations discussing this work.

Cell adhesion and signal transduction in Drosophila. Cavallo, R., Cox, R., Loureiro, J., Kirkpatrick, C., McEwen, D., McCartney, B., Myster, S., Polevoy, G., Rubenstein, D., Peifer, M. ASCB 1998. *Molec. Biol. Cell* 1998 9:461a

"Characterization of a Drosophila homolog of the adherens junction protein p120ctn." Cavallo, R., Myster, S., Audeh, Y., Peifer, M. 40th Annual Drosophila Research Conference, A. Dros. Res. Conf. 40 1999 :304C

"Characterization of the Drosophila homolog of p120ctn, a modulator of adherens junctions." S.H. Myster, R. Cavallo, M. Peifer. 41st Annual Drosophila Research Conference, April 2000. A. Dros. Res. Conf. 41 2000 :435B

"The cloning and characterization of a *Drosophila* homolog of the adherens junction protein p120CTN.", R. Cavallo, S.H. Myster, M. Peifer. Era of Hope, the DOD Breast Cancer Research Program Meeting, Atlanta GA June, 2000.

"Characterization of the *Drosophila* homolog of p120ctn, a modulator of adherens junctions." Myster, S.H., Anderson, C.T., Peifer, M. Characterization of the *Drosophila* homolog of p120ctn, a modulator of adherens junctions. ASCB 2001. Molec. Biol. Cell 2001 12(Suppl.):350a

Degrees supported in part by this work

Ph. D. in Biology Awarded to Rob Cavallo, December 1999, Title: "New Partners for Armadillo in Signal Transduction and Cell Adhesion".

M.S. awarded to Mr. Gordon Polevoy, April 2001, entitled "Mechanisms of Armadillo's roles in signaling and adhesion."

### Appendix 3-- Figures

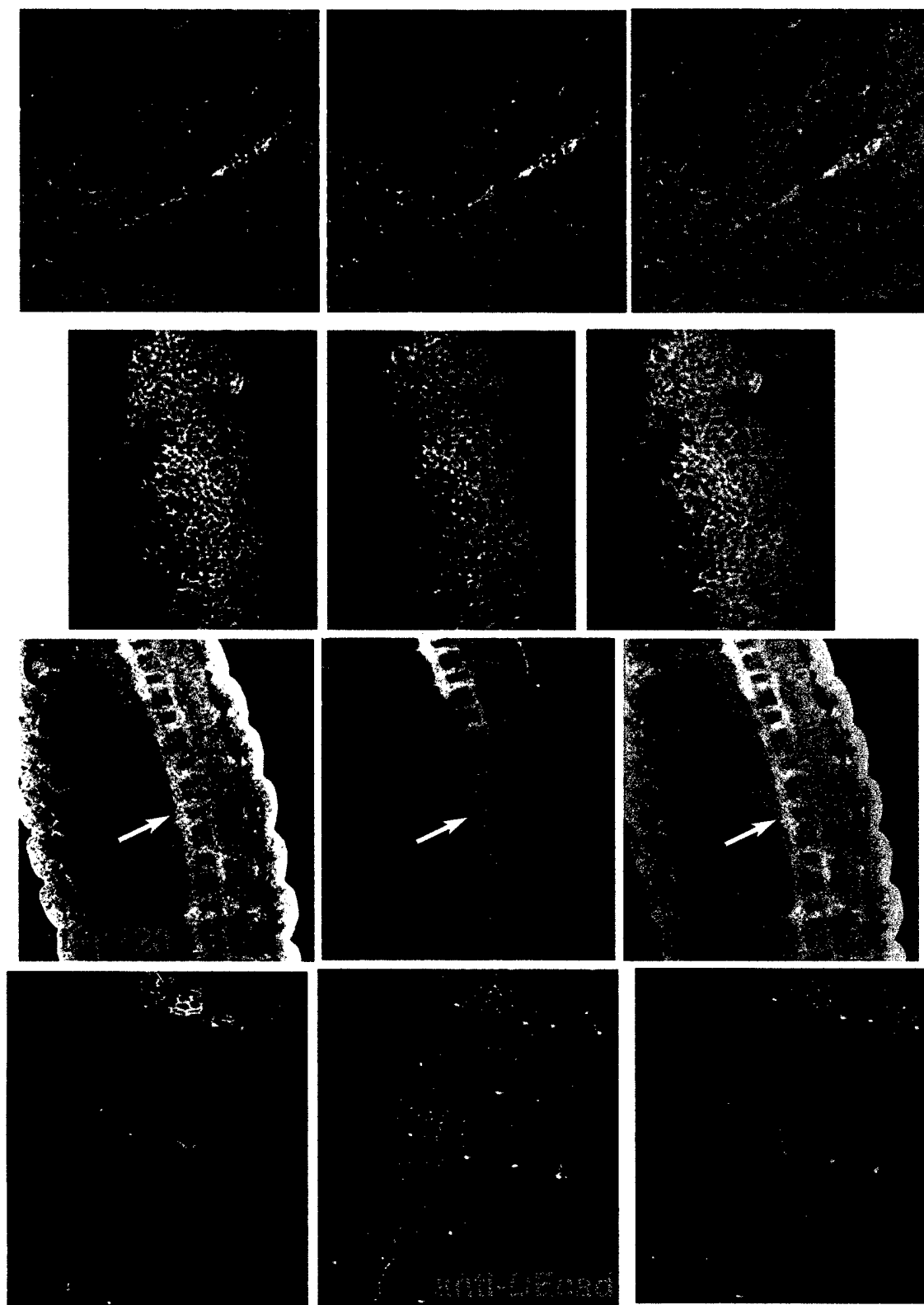
**Figure 1. p120 localization during embryogenesis.** Embryos were fixed and stained with the indicated antibodies. A. Anti-p120 antibodies reveal that p120 localizes to both cell junctions and to the cytoplasm. A stage 9 embryo was double stained with antibodies to p120 (left panel, green in composite) and to the adherens junction protein Armadillo (middle panel, red in composite). The two proteins co-localize to cell-cell junctions. B, C. p120 antibody and antibody to the myc epitope reveal similar patterns of p120 accumulation. Embryo expressing myc-p120 under the control of the ubiquitin promotor double-stained with antibodies to p120 (left panel, green in composite) and to the myc-epitope (middle panel, red in composite). B. Stage 9 embryo showing co-localization to the adherens junctions. C. Stage 15 embryo showing co-localization to the axons of the central nervous system (arrow). D. Antibody to the myc-epitope specifically recognizes myc-p120, which co-localizes with the junctional protein E-cadherin. Stage 10 embryo expressing UAS-driven myc-p120 in alternating body segments, using the paired-GAL4 driver. The embryo was double-labeled with antibody to the myc-epitope (left panel, red in composite) and antibody to DE-cadherin (middle panel, green in composite).

**Figure 2. p120-GFP localization during embryogenesis.** A-F. Embryos expressing p120-GFP were imaged live, using a Perkin-Elmer Spinning disc confocal microscope. A. Cellularizing embryo showing that p120-GFP localizes to cell junctions and to two dots in the cytoplasm (arrow). B. Stage 14 embryo showing p120-GFP localization to cell-cell junctions of both the epidermis and amnioserosa, and its enrichment at the leading edge of the cells migrating during dorsal closure (arrow). C. Stage 15 embryo showing p120-GFP localization to cell-cell junctions of epidermal cells and its enrichment in sense organs of the peripheral nervous system (arrow). D. Stage 14 embryo showing p120-GFP enrichment in the developing tracheal system (arrow). E. Stage 15 embryo showing strong enrichment of p120-GFP in the axons of the developing central nervous system (arrow). F. Stage 15 embryo showing strong enrichment of p120-GFP in the sensory elements of the chordotonal organ (bracket), one of the components of the peripheral nervous system. G. Cellularizing embryo expressing p120-GFP fixed and stained with antibodies to the centrosomal component centrosomin (left panel, red in composite). p120-GFP was visualized by GFP fluorescence (Middle panel, green in composite). The two proteins clearly co-localize to centrosomes.

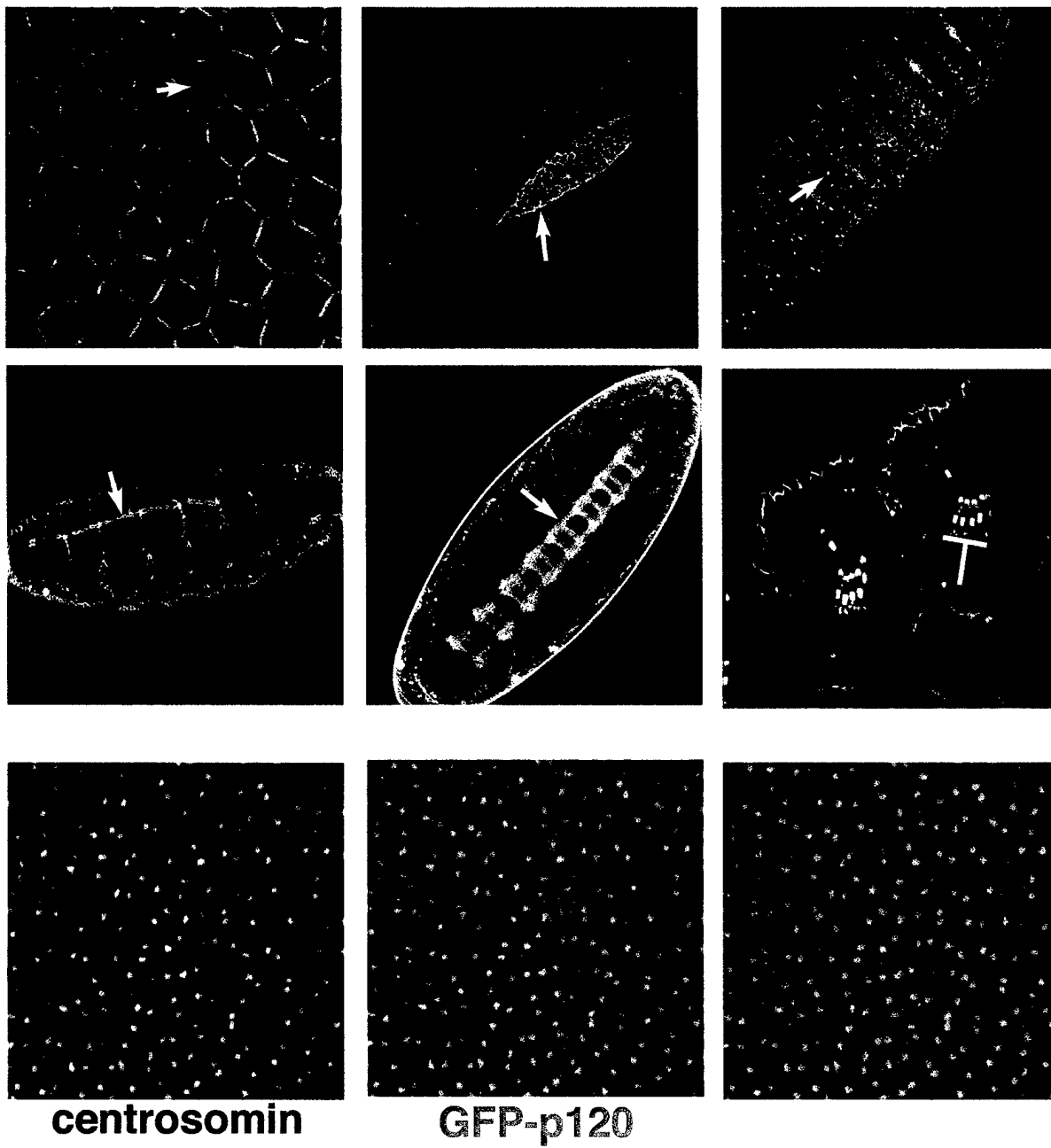
**Fig. 3. The p120 region.** We mapped p120 to region 41C of chromosome arm 2R, at the junction between the euchromatin and heterochromatin. At the top is the genetic map of the region, showing complementation groups we or others have defined in the region. At the bottom is a representation of a portion of the physical map, with coordinates in kilobases, and the open reading frames in the region indicated. The oval indicates the centromere, which is outside of the sequenced region. *p120* represents the second most proximal sequenced gene on 2R, and thus is 68 kilobases from the end of the sequenced region. There are gaps in the sequence of this region, rendering the true distances somewhat uncertain—one of these gaps contains the gene *NippedB*. Between the genetic and physical maps are a set of chromosomal Deficiencies in the *p120* region of chromosome arm 2R. Complementation among Deficiencies and between Deficiencies and alleles from our screen has been carried out, resulting in the positioning of the Deficiencies as shown. We have also carried out PCR experiments to determine which of the open reading frames indicated on the physical map each Deficiency deletes, thus connecting the physical and genetic maps.

Fig. 4. A revised physical map of the 2R heterochromatin. As described in the text, we have used bioinformatics approaches to create a physical contig from the BAC clones and sequenced scaffolds that map to the region. In the center are the scaffolds we have mapped to the region, with their size in kilobases indicated. We are reasonably confident of the order of these scaffolds and in most cases, their orientation. At the top are our suggested placement of sequenced or partially sequenced BAC clones from the region—asterisks indicate a set of tiling path BACs that could be used to complete sequencing of the region. At the bottom are a subset of the sequence tagged sites (STSs) which we used to create the map. We also indicate the approximate size of the gaps between the more distal scaffolds—the size of the gaps between the proximal scaffolds are more difficult to estimate. .

**Fig. 1 Localization of p120 during embryogenesis**



**Figure 2. p120-GFP localization during embryogenesis**



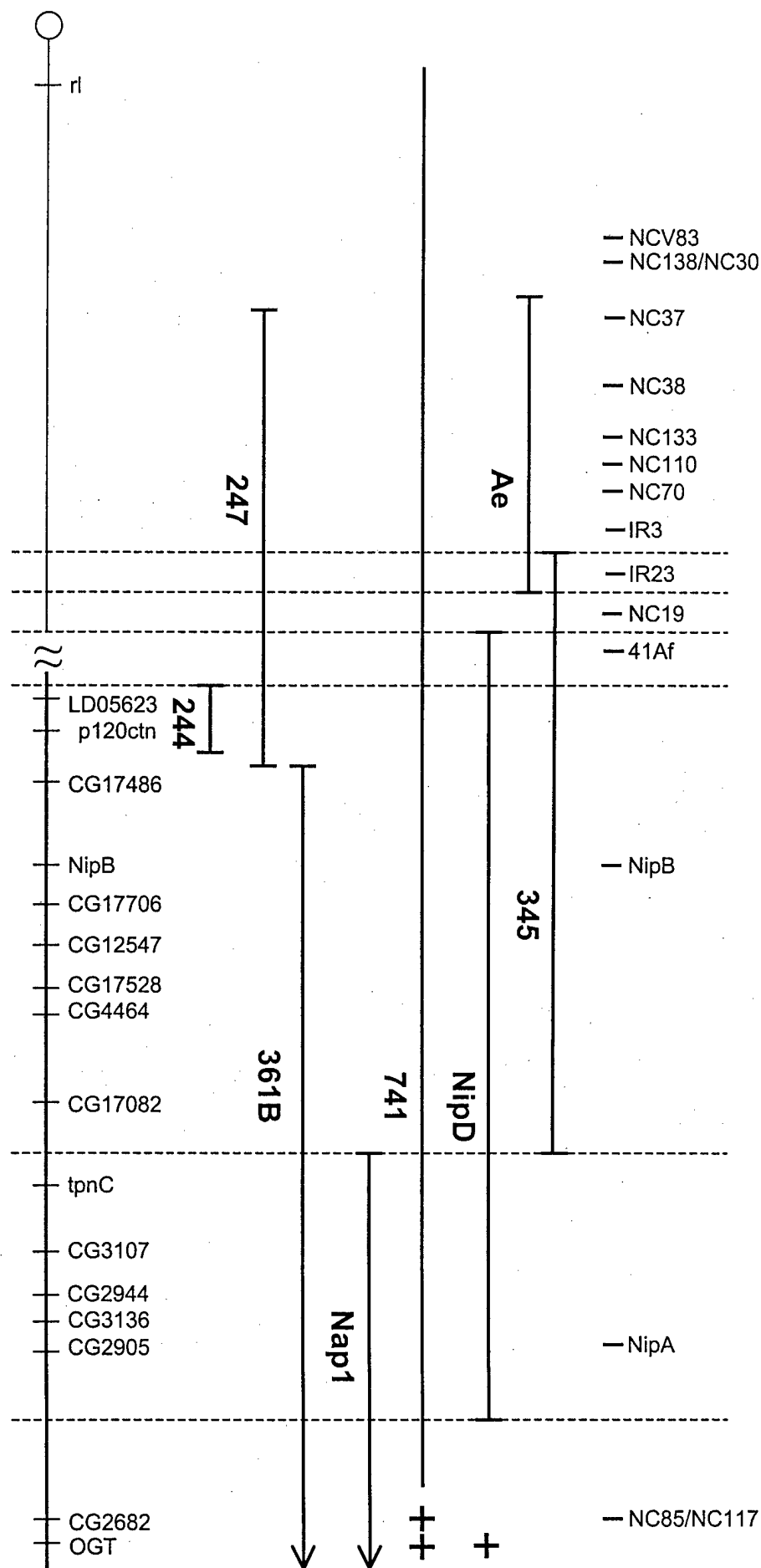


Fig.3

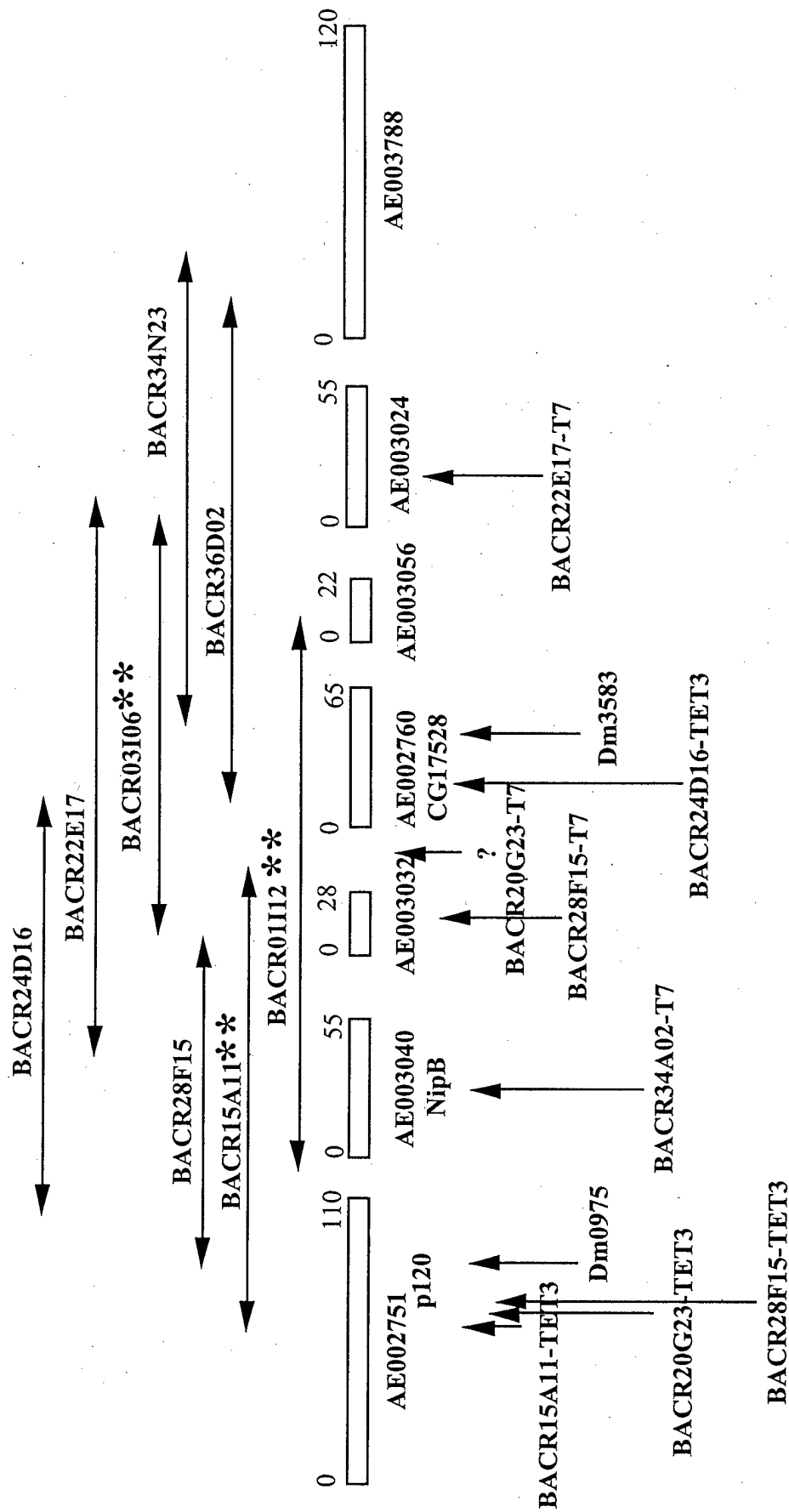


Fig. 4